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Chapter 6

Reinvestigation of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) Sex Pheromone for Improved Attractiveness and Greater Specificity

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Additional information is available at the end of the chapter

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Abstract

Cactoblastis cactorum (Berg.) is recognized as an invasive species in the Caribbean, the United States, and Mexico. Prior work using hexane extracts of sex glands showed that the sex pheromone of this species has 54% of (Z, E) -9.12 -14: acetate, 42% of (Z, E) -9.12 -14:OH and 4% of Z9-14: Ac. Although traps baited with this mixture are effectively to attract males of the cactus moth, it is necessary to determine whether the pheromone can be optimized and to determinate if female diet may impact pheromone composition. Experiments with insects were made at the USDA-ARS Crop in Tifton, Georgia, where there is a colony maintained on cactus and another on an artificial diet. Solid-phase microextraction (SPME) was used to collect pheromones in the headspace above a single calling female and by rubbing the excised female sex gland with SPME fibers. Rubbing the gland directly with SPME fiber revealed that the pheromone consists of the compounds cited above plus Z9-14:Ac. With dynamic aeration and capture of volatiles with fiber only captured two compounds. In addition, our results indicated that natural or artificial diet does not influence the composition of the sex pheromone.

Keywords: cactus moth, sexual pheromone gland, cactus, Mexico, rubbing gland

1. Introduction

The South American cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), was used success fully for biological control for several invasive *Opuntia* species around the world

[1–3]. However, it is also recognized as an invasive species in the Caribbean, the south eastern USA, and Mexico. This followed its release on some Caribbean islands beginning in 1957 [4], its subsequent spread to most other islands in the Caribbean, its attack of most of the 20 *Opuntia* species native to the region [5], and its eventual detection in the Florida Keys in 1989 [6, 7]. *C. cactorum* now threatens more than 80 species of the economic and ecological native and cultivated *Opuntia* species in the United States and Mexico [8, 9, 3, 10]. As *C. cactorum* spread throughout most of the Florida peninsula and along the Atlantic coast to South Carolina and the Gulf coast of Louisiana [11], Mexico developed an awareness/preventative campaign which included monitoring and sampling in commercial and wild areas [12].

Lures used in this work were formulated with the putative sex pheromone components elucidated by following solvent extraction of excised sex glands [13]. Pheromone components were a three-component blend of 54% (Z, E) -9.12 tetradecadien-1-ol acetate (Z9, E12-14: Ac), 42% (Z, E) -9.12 tetradecadien-1-ol (Z9, E12-14: OH) and 4% Z9-tetradecen-1-ol acetate (Z9-14: Ac). This blend when formulated on rubber septa was found to effectively attract male moths; however, changes in the ratio of these components had little effect on lure efficacy. Also, Heath et al. [13] reported that in wind tunnel bioassays the percentage of male moths landing on the odor source was higher when live females were used as a lure than for any of the synthetic pheromone blends. They surmised that, even though the lure was effective in monitoring populations of *C. cactorum* males, identification of additional components was needed to elucidate all the pheromonal components used by *C. cactorum* females.

Although there are some concerns about the efficiency and selectivity of this sex pheromone lure, it was used successfully to detect and monitor populations of the cactus moth in the United States and Mexico. This lure was especially helpful in detecting and monitoring *C. cactorum* populations during the outbreaks of *C. cactorum* in wild cactus (*Opuntia strictadelini*) in Isla Mujeres on August 10, 2006, and in Isla Contoy on May 4, 2007, both located in Quintana Roo, Mexico. With actions implemented immediately by the Mexican phytosanitary authorities, populations were eliminated through the use of trap monitoring, host plant removal, sanitation, and the sterile insect technique [14]. Eradication was declared for *C. cactorum* in Isla Mujeres [15] and Isla Contoy [16] in 2009 in accordance with the model proposed by Tassan et al. [17]. Mexico continues to monitor throughout the country for early detection of new incursions.

While the usefulness of the commercial pheromone has been demonstrated, several factors suggest that there may be missing components or that the blend of components could be optimized. As noted, Heath et al. [13] found that changes in the ratio of the three components in the pheromone blend had little effect on lure efficacy. Also, they found that in wind tunnel bioassays the percentage of male moths landing on the odor source was higher for live females than for any of the synthetic pheromone blends. Another concern is that, unlike traps baited with virgin female moths, traps baited with the synthetic pheromone lure often capture large numbers of "non-target" moth species. Not only does the capture of non-target species suggest a lack of specificity for the pheromone lure, it creates the need for additional labor to examine survey traps for the presence of *C. cactorum* males when the traps contain numerous other moth species.

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In this study, we re-examined the composition and proportion of sex pheromone components of *C. cactorum* by analysis of pheromone gland volatiles using solid phase microextraction technique (SPME). This was done by capturing volatiles within the headspace surrounding calling females and by direct contact of SPME fibers with excised moth sex glands. SPME has been used in this manner to elucidate moth pheromone chemistry in numerous studies [18, 19]. We also used SPME to compare the sex pheromone produced by female moths reared on a meridic diet devoid of Opuntia plant components to the sex pheromone produced by female moths reared on Opuntia cladodes. Overall objectives of this study were to improve the efficacy of the sex pheromone lure, to reduce the number of nontarget moths collected in traps baited with the synthetic sex pheromone, and to compare the quality of sex pheromone produced by female moths reared on host plants and meridic diet.

2. Materials and methods

2.1. Insects and rearing

All *C. cactorum* used in this study originated from the laboratory colony at the USDA-ARS Crop Protection and Management Research Unit Laboratory, Tifton, Georgia. This colony was established from multiple collections of *C. cactorum* larvae from infested *Opuntia* spp. along the Florida Gulf Coast during 2002 and 2004, and from nearly 10,000 eggs collected from *Opuntia* spp. plantations near Craddock, South Africa, and shipped to Tifton, Georgia in 2002. Insects were reared continuously either on a meridic diet devoid of Opuntia plant material [20] or on *Opuntia ficus-indica* cladodes using the protocols described by Marti and Carpenter [21]. Diet-reared and Opuntia-reared pupae were separated by gender and placed in separate containers under the same condition until emergence.

2.2. Collection of volatiles with SPME

To sample headspace volatiles we used 35 mm polyethylene film containers with an orifice in the center of the lid of 2 mm in diameter. In this hole was inserted a rubber septum 10 mm outside diameter (Sigma-Aldrich Z553921) with the large opening to the outside. Within this container was placed a female cactus moth 2–4 days of age 1 h before the calling period. At the calling period, the metal sheath of the SPME assembly was inserted through the rubber septum once inserted and the fiber (Supelco 57300-U) was extended and held in the exposed position by 10 min. The fiber was then withdrawn into its sheath and the assembly placed in 15-mL glass centrifuge tube sealed with a Teflon-lined screw cap. The fiber was then transported to the analytical laboratory for gas chromatography-mass spectrometry (GC-MS). There were a total of five repetitions. Fibers were preconditioned by holding in the 250°C injection port of a gas chromatograph for 1 h prior to each use.

2.3. Collection of volatiles by SPME fiber contact with pheromone glands

Sex pheromone glands were obtained from 2- to 4-day-old females after 8–9 h PM after lights were turned off. Females were most frequently observed to take a calling posture at this time.

The tip containing the pheromone gland was excised when it was exposed with fine tweezers and rubbed repeatedly with the exposed SPME fiber SPME for 15 s. The fiber was withdrawn into its metal sheath and stored in a sealed centrifuge tube prior to GC-MS analysis: Frerot et al. [22] were the first to report the use of this approach to lepidopteran pheromone analysis.

2.4. GM/MS

Each SPME fiber was thermally desorbed in the inlet of a Thermo Finnigan DSQII (San Jose, CA, USA) gas chromatograph-quadrupole mass spectrometer. The inlet temperature was 220°C with desorption for 1 min during split less injection. The instrument's oven was fitted with a 30 × 0.25 mm (i.d.) J+W DB-WAX-fused silica capillary column (Agilent, Santa Clara, CA, USA). The column liquid film thickness was 0.25 μm. Helium carrier gas flow was maintained at 2.0 mL min^{-1}. Following injection, the initial oven temperature, 60°C, was held for 1 min. The temperature was then increased to 240°C at 5°C min^{-1} and held for 4 min. Ionization use, the mass spectrometer was tuned to meet manufacturer performance criteria for perfluorotributylamine. Authentic standards of a mixture of (Z9,E12)-tetradecadien-1-ol acetate, (Z9,E12)-tetradecadien-1-ol, (Z9)-tetradecen-1-ol, and (Z9)-tetradecen-1-yl acetate obtained from Bedoukian (Danbury, CT, USA) were dissolved in methylene chloride analyzed by split less injection into the GC/MS to confirm structural assignments.

2.5. Chemicals

(Z9, E12)-tetradecadien-1-ol acetate, (Z9,E12)-tetradecadien-1-ol and Z9-tetradecenyl acetate were obtained from Bedoukian Co. The purity of compounds was as follows: Z, E-9, 12-14: Ac 93% (Cat Bedoukian P6050-93), Z, E-9, 12–14: OH 93% (Cat Bedoukian 6051 93) and Z9-14: Ac 95% (Cat Bedoukian P6030-95).

2.6. Lure formulation

Rubber septa with a 10-mm outer diameter (Sigma-Aldrich Cat Z553921) were loaded with one milligram of different proportions of the components of the cactus moth sex pheromone. Each septum was held for 24 h in a fume hood to allow evaporation of the solvent. Septa containing the commercial pheromone (Suterra, Inc.) or empty septa were used as controls.

2.7. Field tests

Field tests were conducted in Pampa Muyoj, in Argentina from 1 to 25 March 2011 within a 100-ha cactus plantation. Baited Pherocon 1-C Wing traps (Trécé) were used in all field tests. Tests were conducted during peak flight periods. Treatments were traps baited with various release rates and/or ratios of the putative pheromone components found in this study and tested in comparison with traps baited with commercial pheromone and two virgin females (**Table 1**). Females were 1–2 days old when placed in the traps and were replaced after each sample period. Synthetic lures were replaced after 2 weeks. A number of males captured were determined every 3–4 days. The ratios of a components blend were tested at doses of 1 mg per septa. Trap location within a replicate was randomly selected and randomized each day. Traps were wired onto cactus pads 0.5–1.0 m above ground along cactus rows. Each replicate

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set was separated by at least 20 m, and each trap within a replicate was 3–5 m apart. The number of males captured in each trap was determined daily. All treatments were replicated five times. Control traps were baited with septa treated with the same amount of hexane and in some experiments, we used virgin females 2–4 days old.

2.8. Data analysis

All counts were converted to a number of insects per trap, and data were analyzed using analysis of variance (ANOVA), with the minimum variance unbiased quadratic estimation PROC MIXED MIVQUE0 [23]. MIVQUE0 provides reliable estimates of parameters for data with a non-normal distribution, large numbers of zero values, and unequal variances. Weekly capture data from each trap are used as replicate data for individual traps in the statistical analyses. Results on the graphs are presented as means (±SEM) across all trapping periods.

Treatment	Virgin females	Commercial pheromone	Z,E-9,12-14: Ac	Z,E-9,12-14: OH	Z9-14: Ac	Z9-14: OH	Z9-16: Ac	Tetradecanoic acid
1	*							
2		*	52	44	4			
3			52	44	4			
4			38	44	4	4		4
5			41	56	4			
6			38	44	4	4	4	4
7								
8			60	40				
9			40	60				

Table 1. Combinations of the sex pheromone components of the cactus moth, evaluated in the field at Pampa Muyoj, Argentina, 2011.

3. Results and discussion

3.1. Pheromone identification

Each SPME analysis involved a use of a single calling female. Ion current chromatograms following SPME "headspace" sampling and direct contact with the moth sex gland are shown in **Figure 1**.

The ion used to construct these chromatograms, m/z = 67+, is the base peak of spectra of the mono and di-unsaturated alcohols and acetates that were detected [24]. The four compounds positively identified by comparison of retention times and full scan spectra to authentic

standards were (Z9, E12)-tetradecadien-1-ol acetate, (Z9, E12)-tetradecadien-1-ol, (Z9)-tetradecen-1-ol, and (Z9)-tetradecen-1-yl acetate. Chromatographic data showed that two sampling methods provided matching results. Means of the percent composition of pheromones identified by headspace and contact sampling were 45, 50, 3, and 2 and 47, 47, 3, and 3%, respectively (**Figure 2**). Significant differences were not indicated when peak to peak comparisons were made.

In addition, SPME results were in close agreement with results reported by Heath et al. [13]. Their data which were obtained following solvent extraction of sex glands closely match our findings using SPME sampling (**Figure 2**). The minor exception was that we detected (Z9)-tetradecen-1-ol. This compound was not reported by Heath et al. [13].

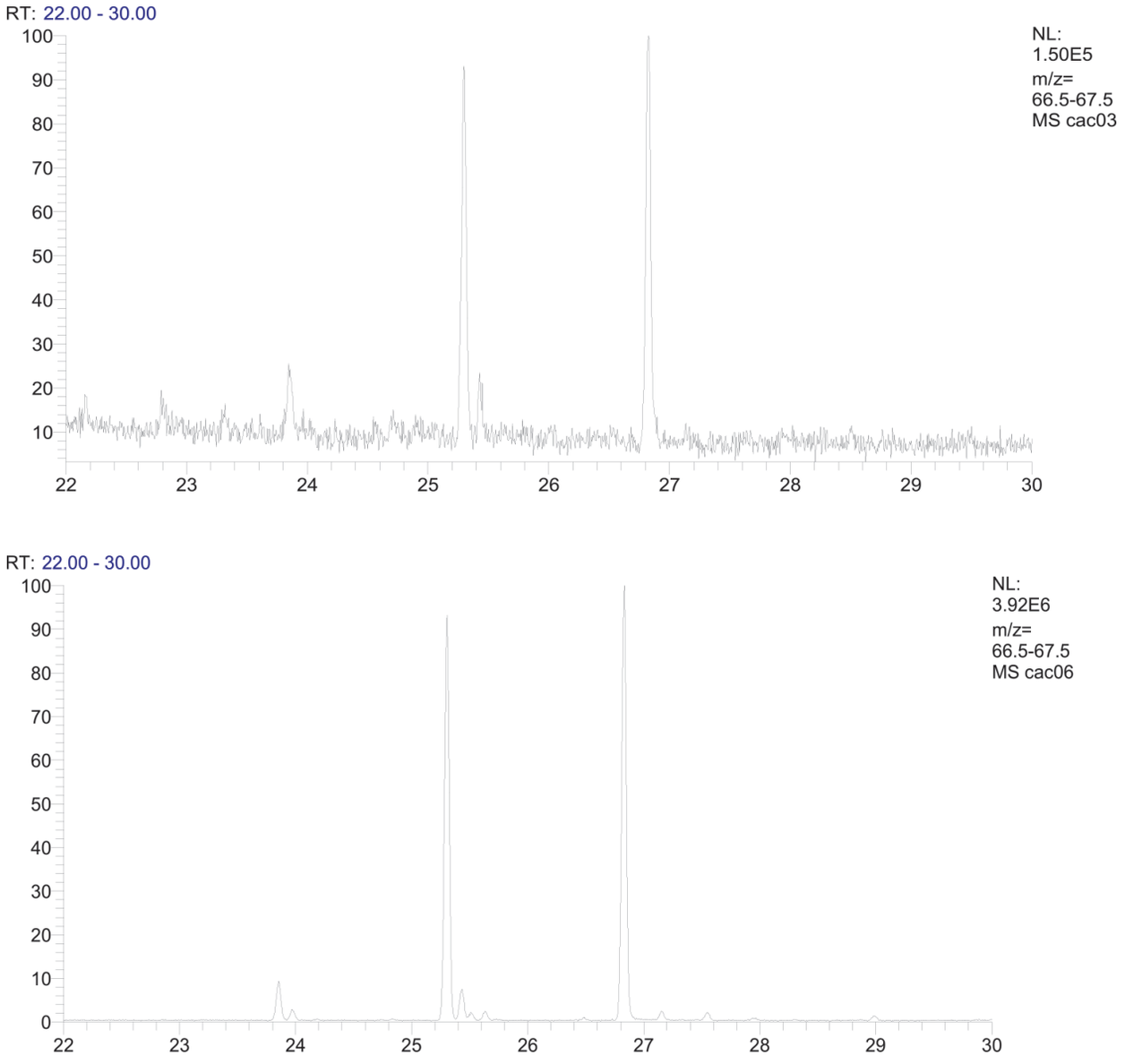

Figure 1. Ion current chromatograms (m/z = 67+) using SPME to sample the headspace above a single cactus moth calling female and by direct contact of the SPME fiber with a single moth sex gland.

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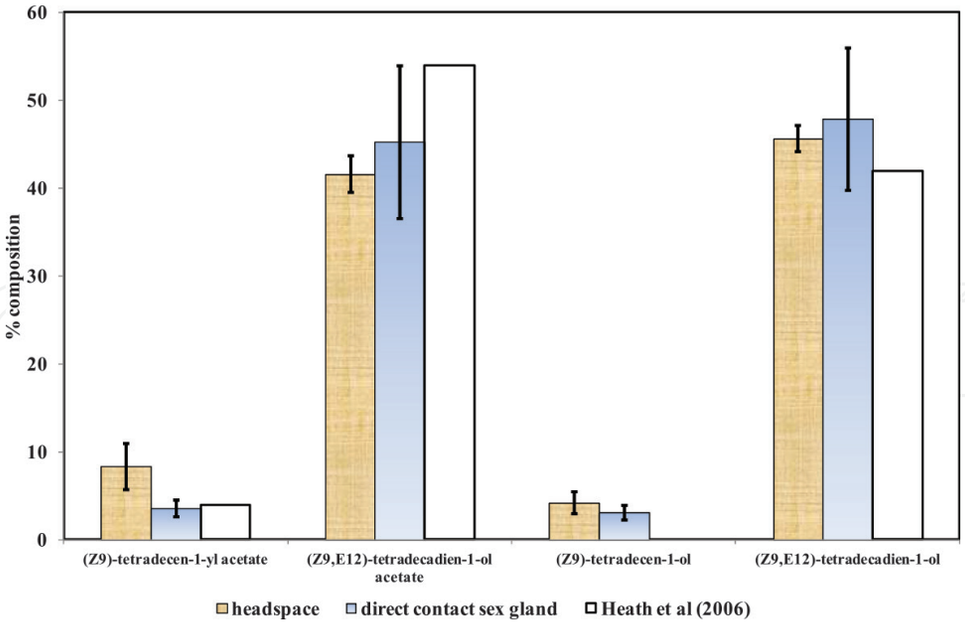

Figure 2. Cactus moth pheromone percent composition by SPME sampling of headspace above single calling females (n = 3) and by direct contact of SPME fibers with a moth sex gland (n = 3) compared to results obtained flowing solvent extraction of sex glands reported by Heath et al. [13].

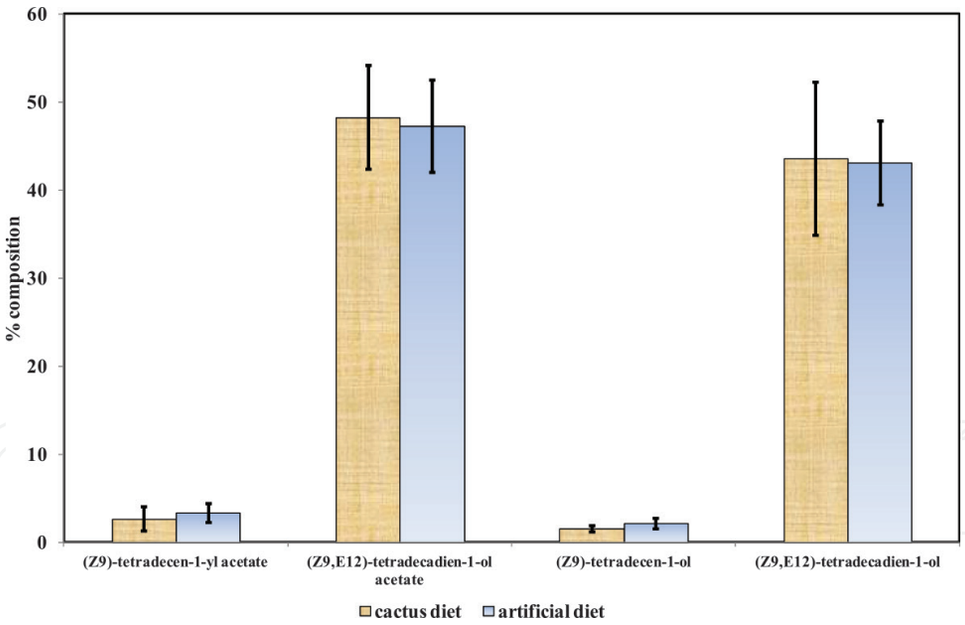

Figure 3. Percent composition of pheromone in cactus moths reared on artificial and cactus diets measured by SPME contact of sex glands of single calling females.

Finally, our analyses did not indicate that diet had an impact on pheromone composition. Only minor differences were detected when pheromones from moths raised on the artificial and cactus diets were analyzed and the percent composition was not significantly different (**Figure 3**). Dietary fatty acids commonly serve as precursors for pheromone biosynthesis in

Lepidoptera [25]. This suggests that the diets used in our study both provided these dietary precursors and as a result differences in pheromone composition were not found.

The results of catches of male cactus moth and other lepidopteran species with different blends and virgin females in Pampa Muyoj, Argentina, are shown in **Figures 4** and **5**. The number of males captured of the cactus moth by the various treatments was statistically different (F = 2.56, P value = 0.02). Treatment of only two compounds (Z9-E12-14: Ac-E12 and Z9-14: OH, T8) in the ratio of 60:40 captured a greater number of the male moths, followed by the reverse ratio of 40:60 (Z9-E12-14: Ac and Z9-E12-14: OH, T9). It is clear that using fewer bait compounds this will be cheaper long as you maintain the same efficiency that comparable lures. Virgin females (T1), the commercial pheromone (T2), commercial pheromone prepared in our laboratory (T3) and remaining mixtures caught similar numbers of males but different from the control (**Figure 4**).

Adding Z9-14: OH (T4) did not improve the efficiency of the bait. A similar effect was noted with the addition of Z9-16: Ac and tetradecanoic acid (T4 and T6), respectively. It is clear that the combination of di-unsaturated acetate and the corresponding alcohol play a key role in the communication system of this species, as in *Copitarsia decolora*, showing a similar effect with only two compounds [26, 27]. Treatment number nine had lower capture of males (although not statistically different from T8). One possible explanation is that the perception range of *C. cactorum* male is relatively broad as shown in *C. decolora* [27]. In this experiment, and in others, it became evident that the pheromone produced in our laboratory with proportions similar to the commercial pheromone (Suterra), captured a smaller number of males (although not statistically different with the commercial). Further chemical analysis showed that both septa are equal, the proportions are similar, but in the septum with the commercial pheromone, we found the anti-oxidant 2, 6-Di-tert-butyl-4methylphenol. This compound may prolong the lifetime of the compounds (date). A similar effect was observed in the mixture of 60:40, perhaps with the addition of the antioxidant would be more efficient in capturing males.

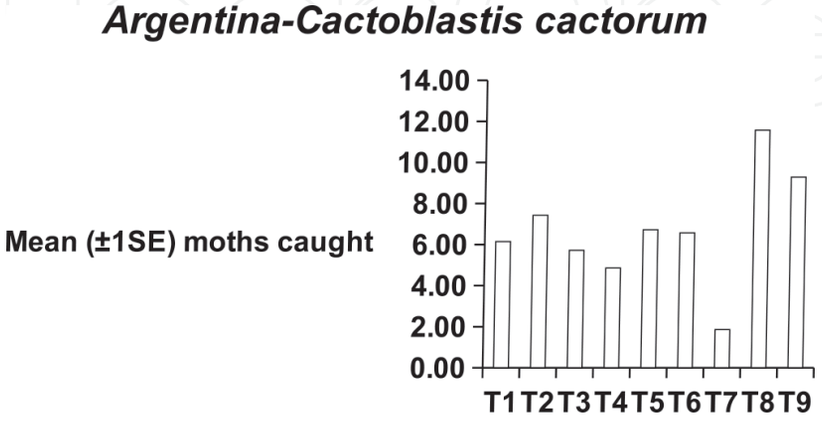

Figure 4. Average (mean ± SEM per trap) of catch per trap of male cactus moth, baited with different blends and virgin females, Muyoj Pampa, Argentina, 2011. Same letters above the bars indicate no statistical difference.

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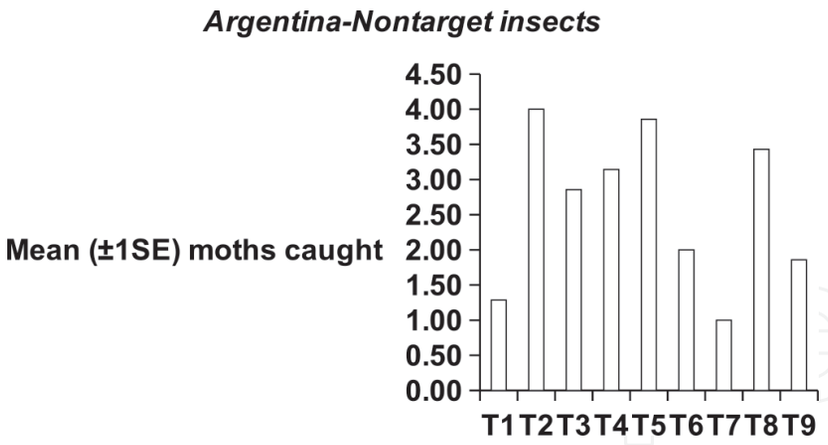

Figure 5. Average (mean ± SEM per trap) trap catches from non-target species, baited with different blends and virgin females, Pampa Muyoj, Argentina, 2011. Same letters above bar indicate no statistical difference.

An important aspect in the use of pheromones is to avoid catching nontarget species [28, 29]. Reducing the capture of nontarget insects increases the efficiency of the trap, reduces the potential impact on endemic species [30], and facilitates inspection of the trap [31]. In the present study, the evaluated mixtures showed no statistical difference in the capture of non-target insects (F value = 1.45, Pr > f = 0.2030, **Figure 5**). Apparently, the evaluated mixtures did not influence decisively in some of the treatments on insects caught. Perhaps, a contributing factor in that no significant difference is due to the low number of non-insects captured by treatments. Probably the type, color of the trap, and the presence of more compounds (T2–T6) could influence the capture of these species. Most nontarget Lepidoptera captured was identified to family. These corresponded to the family Pyralidae and Noctuidae who share with the cactus moth at least one of the compounds tested and that could influence the capture of nontarget males captured [32].

Sex pheromone traps are an important tool for monitoring activity of cactus moth in the Mexican border, and interpretation of data derived from these traps is important for making pest management decisions. Understanding factors that may affect interpretation of data are important in efforts to design better baits and optimize efficiency of monitoring efforts. Bait designs with low capture efficiency pose the risk of underestimation of pest presence and, thus, the unexpected pest introduction. Conversely, designs that are overly attractive to insects can cause inefficiency of monitoring efforts due to saturation by nontarget insects (such as other lepidopterous species) [33]. Optimally, traps used for efficient pest monitoring should be attractive to pests while being unattractive to nontarget species. For cactus moth, the 60:40 mixture data indicate that this is preferable to other sexual pheromone component combinations.

4. Conclusions

The sex pheromone of cactus moth is composed of (Z, E) -9.12 tetradecadien-1-ol acetate (Z9, E12-14: Ac), (Z, E) -9.12 tetradecadien-1-ol (Z9, E12-14: OH) and Z9-tetradecen-1-ol acetate

(Z9-14: Ac). Rubbing the gland directly with SPME fiber was an appropriate technique for recovering sexual components of sex gland females. Our results indicate that natural or artificial diet does not influence the composition of the sex pheromone. Of the eight mixtures evaluated in the field, more moths were captured with binary mixtures of the di-unsaturated acetate and di-unsaturated alcohol in 60:40 proportions. Using two-component mixtures as bait will likely make traps less expensive while providing capture efficiencies that are equal to or greater than commercial traps that are currently available that use four components and therefore more expensive. Currently, the mixture of the main compounds in 60:40 proportions is used in 1400 wing traps that are changed and checked every month along the Gulf of Mexico to detect the entry of the cactus moth to Mexico. In the near future, the next step will be to study the volatiles of *Opuntia* species and determine if the female of the cactus moth uses them to find their host plant. Thus, we would have a pheromone for capturing males and an attractant for capturing females.

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